Leptin and Reproduction
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Leptin, a hormone secreted from adipose tissue, plays an important role in reproductive physiology. It has been shown to stimulate the reproductive system by rescuing the sterility of leptin-deficient mice and advancing the onset of puberty in normal mice. Although leptin is not critical for the biology of pregnancy in mice, its ability to reduce food intake is blunted in midgestation suggesting that late pregnancy may be a leptin-resistant state. Modifier genes originating from the Balb/cJ genetic background profoundly alter the sterile-obese phenotype of ob/ob mice by reducing their obesity and stimulating their reproductive system despite the absence of leptin. The mechanism of leptin’s action on the reproductive system remains to be determined but is likely to be mediated by multiple factors.

Key Words: leptin, adipose tissue, reproductive physiology, sterility, pregnancy, leptin-resistant state, ob/ob gene

Introduction
Prior to the cloning of leptin, the sterility of leptin-deficient (ob/ob) obese male and female mice was only marginally investigated. In the 1950s, however, Priscilla Lane addressed this issue by parabiosing castrated normal and ob/ob mice with intact normal or ob/ob female mice. The aim of this experiment was to disrupt, in one parabiont, the negative feedback at the pituitary–gonadal axis to stimulate oversecretion of follicle stimulating hormone (FSH), which would then circulate to the other female parabiont causing a corresponding degree of ovarian hypertrophy. Weights of the ovaries in the intact parabiont would reflect the relative quantity of FSH release from the castrated parabiont. Briefly, Lane found that castrated ob/ob mice elicited less ovarian hypertrophy than did normal mice suggesting that ob/ob pituitaries were not as competent as normal mice in secreting FSH. Furthermore, when ob/ob females acted as the recipient partners, they showed vaginal cornification and estrous cyclicity demonstrating that their ovaries were functional despite their obesity. Although Lane found no evidence for the correction of obesity as a result of these parabiosis experiments, the lack of effect on body weight was probably due to the low circulating levels of leptin from the normal mouse in the parabiotic union. Ten years later, Coleman parabiosed leptin-overexpressing db/db mice to leptin-deficient ob/ob mice and proposed that a circulating factor in db/db mice could correct the obesity of ob/ob mice. In turn, the db/db mice were postulated to be resistant to the effects of this circulating factor owing to a mutation in the receptor of this factor. Because of these enlightening experiments, which were largely vindicated by recent findings, the earlier parabiosis experiments of Lane and colleagues on the reproductive aspect of ob/ob mice were overshadowed. Unfortunately, the seminal parabiosis experiments of Coleman did not address any reproductive question.

Reproduction in Leptin-deficient ob/ob Mice
The ob/ob mouse has long been known to be sterile. Administration of reproductive hormones such as FSH, luteinizing hormone (LH), and relaxin to ob/ob females was successful in inducing ovulation, pregnancy, and delivery, suggesting that a defect in reproductive hormones secretion was the basis of their infertility. Maintenance of the ob/ob and db/db strains by transplantation of their ovaries into normal female hosts suggested that the reproductive defect of these obese mice was central and not peripheral as implied by Lane’s parabiosis experiments. The relatively recent discovery of the nonsense mutation R105X in the ob gene of ob/ob mice revealed that these mice were deficient in the production of a secreted hormone that has been termed leptin. Recombinant expression of leptin allowed its purification in quantities large enough for rescuing experiments in ob/ob mice. Continuous injections of recombinant leptin into ob/ob mice resulted in drastic reductions in food intake and fat mass, thus validating its
authenticity and biologic activity.11–14 Whereas most reports concentrated on the obese and diabetic phenotypic features of this animal model, we showed that administration of recombinant leptin to ob/ob mice not only reversed the obesity but had rescuing effects on the reproductive axis as determined by successful mating of ob/ob males and ob/ob females with wild-type mice (Figure 1).15,16 Thus, unlike food-restricted ob/ob mice, which fail to activate their reproductive axis despite weight reduction, leptin-treated ob/ob mice lost weight and could reproduce thereby showing that leptin was capable of activating their defective reproductive axis (Figure 2). Although not measured at the time, rescuing the sterility of ob/ob mice with leptin treatment implied that well known reproductive hormones such as FSH, LH, and estrogens must have modulated the fertility of ob/ob mice.

Because reports have described leptin secretion from the placenta,17–19 we asked whether leptin was critical for the biology of pregnancy. We carried out an experiment in which the strategy was to have a pregnancy completely devoid of endogenous leptin such that any leptin during the pregnancy could be manipulated by exogenous administration.20 After inducing fertility in leptin-treated C57BL/6J ob/ob males and C57BL/6J ob/ob females, we mated them together such that similar to the ob/ob mother, all fetuses would be leptin deficient. At this point, leptin treatment was either discontinued at day 0.5 when the copulatory plug was first detected or extended to 3.5, 6.5, 10.5, or 19.5 days into the pregnancy. The reasoning behind leptin withdrawal was to determine whether leptin was required for implantation and subsequent growth of the fetus and to assess its impact on food intake during pregnancy. The results of this experiment were disappointing in terms of leptin’s importance during pregnancy. We found that irrespective of the time of leptin withdrawal after copulation, ob/ob pregnancies progressed to parturition without leptin (Figure 3). Interestingly, when administered throughout gestation, leptin did not reduce food intake after midpregnancy, suggesting that the late stage of a mouse pregnancy is a leptin-resistant state. We concluded from these experiments that leptin is not critical for pregnancy but that a new pinnacle of pregnancy is the establishment of a leptin resistance, which favors maternal and fetal nutrition.

Impact of Modifier Genes on the ob/ob Phenotype

It is fairly well known that allelic variants may have a profound impact on the progression of a mutant phenotype. These variants encoded by modifier genes are the basis of genetic diversity and can be brought into a phenotype by crossing the mutation of interest onto a different genetic background than its original one. We set up to determine how the Balb/cJ genetic background modifies the sterile-obese phenotype of ob/ob mice, which have been almost solely studied on the C57BL/6J background. We thus generated two new lines of ob/ob mice with respect to their genetic backgrounds. One line was generated via an F2 intercross to yield ob/ob mice carrying a mixed C57BL/6J-Balb/cJ genetic background and the second line was created through 10 successive rounds of backcrosses to produce a congenic ob/ob line virtually on a pure Balb/cJ genetic background. The first cross revealed that during a 15-week mating period, 41% of male F2 ob/ob mice were capable of inducing one to six pregnancies in normal female mice (Figure 4).21 The F2 ob/ob females, however, were incapable of sustaining any pregnancy. Whether this was due to the lack of mating, ovulation, failure of implantation, or insufficient numbers of F2 ob/ob females was not determined. Furthermore, the body weights and adiposities of F2 ob/ob males at 23 weeks of age were significantly reduced when compared with C57BL/6J ob/ob males (Figure 4). Thus, modifier genes from either the Balb/cJ background alone or the interaction of C57BL/6J and Balb/cJ backgrounds act to decrease the obesity and enhance the fertility of male F2 ob/ob mice. We concluded from these studies that the action of these modifier genes on obesity and fertility was independent of leptin because they rescued the reproductive system and attenuated obesity in the absence of leptin. It remains to be determined whether the induction of these genes is contingent upon the absence of leptin, thus providing a compensa-
tory mechanism for a defective leptin pathway or/and whether they can modulate its action.

A genome scan on the F2 males was then undertaken in the hope of identifying chromosomal regions that are associated with particular qualitative traits such as body weight and the number of pregnancies that an F2 ob/ob male could induce in normal females. The genome scan included 123 microsatellites that were spaced on average by 10 cM and which were polymorphic between the C57BL/6J and Balb/cJ strains. When the data were analyzed with the Map Manager quantitative trait-locus program, the most significant correlations were found with body weight, which showed a logarithm of the odds (LOD) score of 5.0 on chromosome 5 at the D5MIT271 locus and a recessive mode of inheritance. Fertility, denoted by the number of pregnancies showed a LOD score of 5.6 on chromosome 1 at the D1MIT459 locus and an
additive mode of inheritance. Both of these values exceeded the LOD score of 3.4 proposed by Lander and Kruglyak22 as threshold for significant linkage. Therefore, it is likely that Balbc/J and C57BL/6J genes in these chromosomal regions significantly influence body weight and fertility of F2 ob/ob mice.

In the second cross, derivation of the Balbc/J congenic ob/ob line revealed that the body weights and adiposities of this new strain of ob/ob mice were drastically reduced compared with those of the classic C57BL/6J ob/ob strain (Figure 5).23 For example, at 25 weeks of age, the body weights of Balbc/J ob/ob males and females had already reached a plateau of approximately 50 g, whereas C57BL/6J ob/ob mice weighed more than 75 g, a whopping difference of 25 g, mostly attributed to adipose mass. Although the immediate culprit might be suspected to be a differential food intake between these two strains, this was not the case. Monitoring of daily and total food consumption for 18 days revealed that both strains of ob/ob mice were ingesting similar amounts of food, thus ruling out food intake as a major contributor for differences in their adiposities. Rather, metabolic differences might account for these effects. We then measured plasma glucose, insulin, triglycerides, and cholesterol levels in fasted and fed ob/ob mice from both strains and found significantly elevated insulin and triglycerides levels in Balbc/J ob/ob mice, only in the fed state. This finding indicated that the Balbc/J ob/ob mice were more diabetic than their C57BL/6J ob/ob counterparts and suggested that possibly their reduced obese state might be secondary to their uncontrolled severe diabetes. This hypothesis was based on the observation that C57BL/Ks ob/ob mice lost weight progressively and died of beta cell exhaustion and pancreatic failure at approximately 6 months of age.24 However, unlike C57BL/Ks ob/ob mice, Balbc/J ob/ob mice live more than a year and do not show any evidence of pancreatic failure despite islet cell hyperplasia. Thus, the modifier genes from the C57BL/Ks and Balbc/J genetic backgrounds have profound but different effects on the progression of a diabetic phenotype caused by leptin deficiency.

On the reproductive side, we found that six out of ten Balbc/J ob/ob males and all four Balbc/J ob/ob females were capable of inducing pregnancies during a 16-week mating period despite their obese state (Table 1). Thus, this congenic line of ob/ob mice could

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**Figure 4.** Fertility and body weights of ob/ob males bred on mixed C57BL/6J and Balbc/J genetic backgrounds. (A) Number of pregnancies that C57BL/6J ob/ob (black bars) and ob/ob F2 (white bars) males induced in lean females. None of the C57BL/6J males could induce any pregnancy, whereas 41% (14 of 34 mice) of F2 C57BL/6J-Balbc/J ob/ob males were capable of inducing one or more pregnancies in normal females. (B) Body weights from 6 to 23 weeks of age of males ob/ob mice maintained on either the C57BL/6J inbred (black bars) or the mixed F2 C57BL/6J-Balbc/J (white bars) background shows that up to 23 weeks of age, body weights of the ob/ob mice from both strains are not significantly different from each other. (C) Body weights of F2 fertile (white bars) and F2 non-fertile (black bars) ob/ob males from 6 to 23 weeks of age demonstrates that the fertile F2 ob/ob males are less obese than their non-fertile ob/ob littermates.

**Figure 5.** Body weights of ob/ob males and females on either the C57BL/6J or Balbc/J genetic backgrounds from 3 to 27 weeks of age. The graphs show that the body weights of Balbc/J ob/ob mice reach a plateau of approximately 50 g as compared with the rampant body weight of ob/ob mice on the C57BL/6J background.
reproduce at a reduced efficiency without leptin. It will be interesting to determine whether the reproductive system of Balb/cJ ob/ob mice that fail to reproduce is more sensitive to exogenous leptin than sterile C57BL/6J ob/ob mice. This would imply that they might have inherited only a subset of modifier genes rendering them genetically susceptible to restored leptin-mediated fertility. Ultimately and although a daunting initiative, the nature of these modifier genes, which might be uncovered by a combination of genetic and genomic strategies, will be of greatest interest in delineating the mechanisms by which they stimulate the reproductive system with or without leptin. In summary, this new strain of Balb/cJ ob/ob mice shows reduced adiposity, severe diabetes, and enhanced fertility. Along the same lines, it is interesting to note that an individual was recently described to have entered puberty and reproductive life at a later age despite a genetic deficiency in leptin production. This apparent paradox can be explained by the existence of modifier genes, which compensate for the loss of leptin by stimulating a delayed onset of puberty. This hypothesis supports our genetic studies on the reproduction of F2 ob/ob and Balb/cJ ob/ob mice and strengthens the stimulatory role of these modifier genes on the reproductive system of humans and rodents.

**Leptin and Puberty**

Extensive literature exists about the association between nutritional state and reproductive maturity. Pioneering studies by Kennedy and Mitra\(^{26}\) proposed that puberty is somehow linked to body weight and more specifically to fat storage, which, as they concluded, is one of the signals responsible for the initiation of hypothalamic control of ovarian function. Further findings by Frisch and McArthur\(^{27}\) suggested that adiposity is a critical determinant for the onset of puberty and the maintenance of menstrual cycles. Conversely, it was found that excessive exercise and subsequent loss of adipose tissue mass (which we now know leads to a reduction in circulating leptin levels) was associated with an interruption of menses.\(^{28,29}\) Therefore, these early findings established a strong association between reproduction and adipose tissue extending the hypothesis that a fat-derived metabolic factor may be responsible for the initiation of reproduction. In light of the discovery that leptin levels correlated with the fat mass and that leptin levels signal via a central hypothalamic circuitry the extent of adiposity of an organism, we reasoned that leptin could be the postulated metabolic factor that links adiposity with reproduction. Thus, we carried out an experiment in which we treated prepuberal normal mice daily with supraphysiologic doses of recombinant leptin in order to trick their neuroendocrine pathways into acting as though they have accumulated adequate energy reserves to turn on their reproductive system. This experiment proved fruitful in advancing the vaginal opening of normal mice by a few days and was accompanied with appropriate maturation of their reproductive tract\(^{30}\) (Figure 6). Similar results were independently obtained,\(^{31}\) thus strengthening the findings from both studies. In addition, it was recently shown that transgenic mice overexpressing leptin by 12-fold showed early signs of puberty.\(^{32}\) Along the same vein, we recently generated a transgenic mouse model that overexpresses leptin by fivefold and investigated its onset of puberty. We found

| Table 1. Fertility of ob/ob Males and ob/ob Females on the Balb/cJ Genetic Background. |
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| **Males** | BW (g) | Age (weeks) | Pregnancies | Age at Mating(s) (weeks) | BW at Mating (g) |
| 1 | 39.7 | 12 | 0 | — | — |
| 2 | 43.0 | 11 | 3 | 21, 22, 26 | 47.5, 48.7, 50.5 |
| 3 | 45.7 | 11 | 2 | 11, 24 | 45.7, 52.1 |
| 4 | 43.3 | 10 | 0 | — | — |
| 5 | 42.9 | 9 | 0 | — | — |
| 6 | 33.1 | 8 | 1 | 13 | 41.7 |
| 7 | 33.5 | 7 | 0 | — | — |
| 8 | 33.7 | 7 | 1 | 18 | 49.8 |
| 9 | 25.9 | 6 | 2 | 8, 19 | 34.3, 45.7 |
| 10 | 25.3 | 6 | 2 | 7, 13 | 30.7, 45.2 |
| **Females** | BW (g) | Age (weeks) | Pregnancies | Age at Mating(s) (weeks) | BW at Mating (g) |
| 1 | 39.0 | 10 | 1 | 11 | 41.7 |
| 2 | 33.6 | 8 | 2 | 13, 39 | 47.3, 63.6 |
| 3 | 29.3 | 8 | 2 | 10, 21 | 38.8, 52.6 |
| 4 | 23.4 | 6 | 1 | 9 | 35.7 |

The body weight (BW) and age of each mouse at the beginning of the mating period and near each mating time are shown.
that on a mixed genetic background, a fivefold overexpression of transgenic leptin did not induce early puberty. However, supplementation of exogenous leptin to these transgenic mice advanced the timing of vaginal opening (Ogus and Chehab, unpublished observations, 2000), suggesting that the effect of leptin on puberty is quantitative and may require more than just fivefold leptin overexpression. This quantitative and threshold model of leptin action on the reproductive system of normal mice is consistent with a previous observation denoting the presence of a postnatal and prepubertal leptin spike of approximately 10 times the basal levels\textsuperscript{33} and with the transgenic early puberty mouse model overexpressing leptin by 12-fold.\textsuperscript{32} However, in the context of the \textit{ob/ob} mouse, which is exquisitely sensitive to leptin, progression to puberty and a reproductive state required considerably less leptin than normal mice as demonstrated by rescuing the fertility but not the obesity of \textit{ob/ob} mice expressing a weak leptin transgene, which expressed only low amounts of leptin.\textsuperscript{34} Leptin therefore plays a significant role in the onset of puberty in the mouse. To investigate whether a similar role for leptin occurs in primates, a search for a leptin surge in monkeys with elevated doses of leptin to assess the impact of such a treatment on their progression to puberty.

**Mechanism of Action of Leptin on the Reproductive System**

Transition from a defective to a functional reproductive system must result in the release of well-known reproductive hormones either directly to the site of action such as gonadotrophin-releasing hormone (GnRH) acting on the pituitary gonadotrophs or into the circulation such as FSH and LH to reach their peripheral target organs. Thus, the endpoint assays for these experiments often rely on the determination of reproductive hormone levels, maturation of the reproductive tract or simply increased fecundity, as exemplified by the stimulation of puberty in normal mice and rescue of sterility in \textit{ob/ob} mice. Therefore, for leptin to stimulate the reproductive system, GnRH, FSH, and LH must be released. Although assays aimed at measuring these reproductive hormones are available in mice, their pulsatory nature makes it difficult to assess their significance when measured only cross-sectionally owed mainly to the small blood volume of the mouse. Therefore, it would be more advantageous to apply genetic strategies that are aimed at the manipulation of a single molecule in a specific pathway. For example, knockout experiments of the MC4 receptor, which is known to mediate the action of leptin on the adipose mass, have resulted in obese but fertile mice.\textsuperscript{37} Thus, the MC4 receptor and its downstream signaling pathways, although critical for body weight regulation, are largely not involved in mediating leptin’s action on the reproductive system. Similarly, knockout experiments of the gene encoding neuropeptide Y,\textsuperscript{38} which was proposed to be an initiator of puberty,\textsuperscript{39,40} did not result in reproductive defects or late puberty in mice ruling it out as a critical factor for the onset of puberty. Thus, the effect of leptin on the onset of puberty must involve other than these factors. One promising approach to unravel factors that may interact with leptin in its stimulatory effect on the reproductive system is to determine the nature of the products encoded by the modifier genes, which are capable of rescuing the sterility of leptin-deficient mice.

**Conclusion**

The role leptin plays in reproductive biology is peculiar and one that transcends our classic knowledge of reproductive hormones, which reach their target organs to elicit hormonal secretions. Rather, leptin may orchestrate and coordinate the reproductive status of an organism by acting as a cross-talk molecule between nutrition and reproduction. As a bioinformant molecule, leptin would monitor the changing nutritional states of an organism by

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**Figure 6.** Acceleration of reproduction in leptin-treated mice. (A) Body weights of saline (PBS) versus leptin-treated prepuberal females at the time the copulatory plug was detected. The graph shows that leptin treated prepuberal mice have a lower body weight at their first mating than saline treated mice of the same age. (B) Percentage and age of plugged mice in the saline-treated (black bars) and leptin-treated (white bars) groups demonstrating an earlier copulation of leptin-treated females. (C) Percentage of deliveries resulting from pregnant mice of both groups shows that despite early copulation, leptin treatment did not interfere with gestation and delivery.
evoking alternative pathways for the reproductive system. How leptin precisely keeps the reproductive system in check remains to be determined, but it is unlikely that it coordinates this critical task single-handedly. Rather, it might recruit additional molecules and hormones to regulate energy metabolism and reproduction, two pathways that are crucial for the survival of the species. Undoubtedly, the years to follow will be exciting for this emerging field and will increase our understanding of the interactions between leptin and the reproductive system.

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